THE EFFECT OF L-ASCORBIC ACID ON ASPARTATE AMINOTRANSFERASE

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1. Introduction

Cytoplasmic aspartate aminotransferase from pig heart contains several subforms, called α, β, γ, etc., according to their increasing electrophoretic anodic mobility, which can be isolated by ion exchange chromatography or electrofocusing [1,2]. The presence of these multiple forms has been demonstrated also in electrophoretograms of single heart homogenates made within a few hours of the death of the animal [1]; it has been also shown that ageing of the isolated subforms in vitro brings about a relative increase of the amount of the more anodic components, in which an inactive mode of binding of the coenzyme prevails [3].

The origin of the subforms is still unclear; it has been tentatively attributed either to minor chemical differences (deamidation of amido groups, oxidation of –SH groups) or to the existence of several possible conformational states of the protein generating different environments of ionizing groups and therefore different p*K*s, [1,3,4,5]. In a recent paper Robinson et al. [6] have demonstrated an acceleration of the rate of deamidation of both a model peptide and a protein, human transferrin on addition of L-ascorbic acid.

In order to get more information on the origin of subforms we examined the effect of L-ascorbic acid on aspartate aminotransferase and we observed changes of the enzyme properties similar to those seen on ageing.

2. Experimental and results

The subforms of aspartate aminotransferase were separated by chromatography as described by Martinez-Carrion et al. [1] except for minor modifications previously reported [7]. Starch gel electrophoresis at pH 7.5 was performed according to Banks et al. [3]. Catalase was a Sigma product, while bovine superoxide dismutase was kindly provided by Dr G. Rotilio.

Samples of the chromatographic fraction of aspartate aminotransferase which contained essentially the α-subform with a small amount of β, were incubated at a concentration of 20 mg/ml, 37°C, in 0.1 M phosphate buffer, pH 7.4 and in the presence of 0.01M L-ascorbic acid, for different periods of time.

The modifications induced by this treatment in the electrophoretic behaviour of the protein (compared with the electrophoretic properties of the mixture of the natural subforms) are shown in fig. 1.

If the incubation is performed in 0.1 M sodium acetate buffer, pH 5.4, the production of the more anodic components, although still clearly detectable, is markedly slowed down.

The formation of these subforms is also inhibited by the presence in the incubation mixture of catalytic amounts of superoxide dismutase and catalase.

Incubation with L-ascorbic acid brings about spectral changes in the near UV region. The spectral changes are reported as a function of the time of incubation in fig. 2. The spectra were obtained by diluting the enzyme with 0.1 M sodium acetate buffer, pH 4.8, and subtracting the absorbance of a solution of L-ascorbic acid incubated in the absence of enzyme. After 24 hr of incubation the absorbance at 430 nm, which monitors the fraction of coenzyme bound in the active mode [1], has decreased to 64% of that at time 0, with a parallel increase in the absorbance in the region below 350 nm. The activity of the same sample is reduced to 67%, which is in good agreement

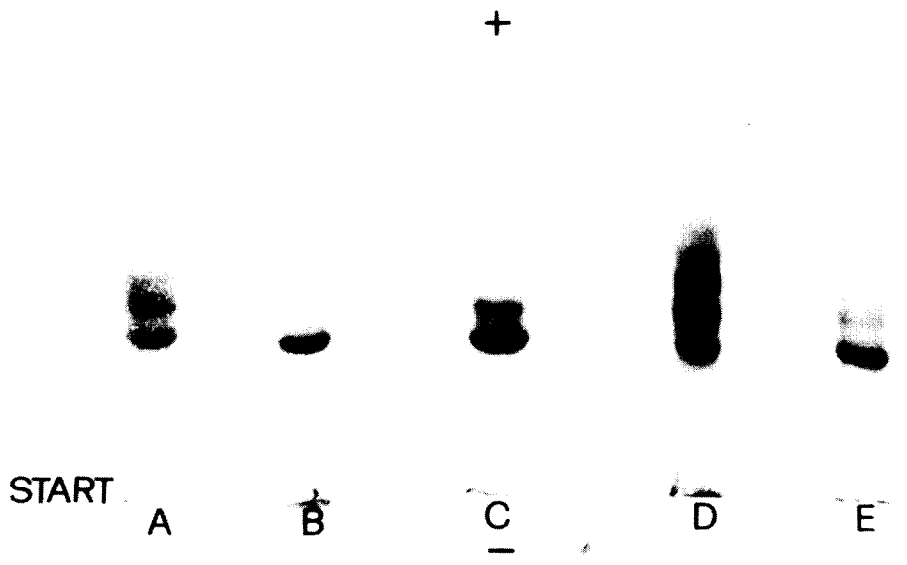

Fig. 1. Starch gel electrophoresis of native and L-ascorbic acid induced multiple forms of aspartate aminotransferase. A: Aspartate aminotransferase before separation of multiple forms; B: α-aspartate aminotransferase; C: α-aspartate aminotransferase incubated for 3 hr with L-ascorbic acid; D: α-aspartate aminotransferase incubated for 7 hr with L-ascorbic acid; E: α-aspartate aminotransferase incubated for 7 hr with ascorbic acid in the presence of 1×10^{-6} M catalase and 1×10^{-6} M superoxide dismutase.

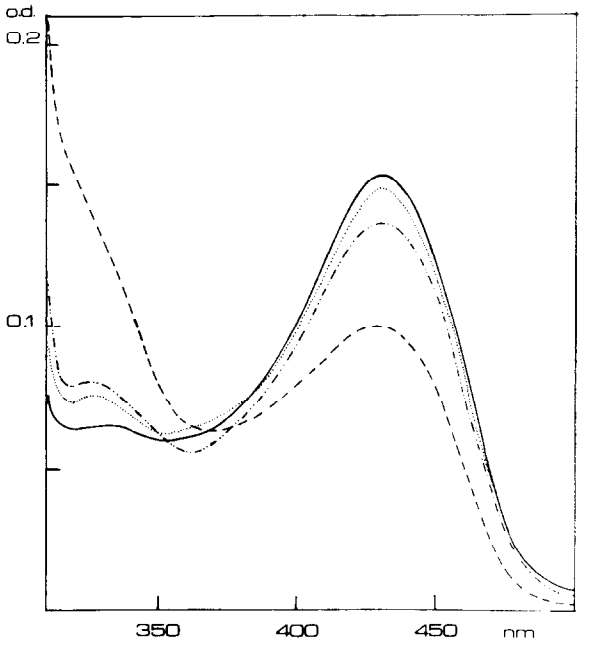

Fig. 2. Absorption spectra of α-aspartate aminotransferase incubated with L-ascorbic acid: ———, incubation time = 0; · · · · · · ·, incubation time = 5 hr; – · · – · · – · ·, incubation time = 9 hr; – – – – – –, incubation time = 24 hr.

with the above reported decrease in absorbancy at 430 nm. Control experiments showed that the enzyme incubated in identical conditions but in the absence of L-ascorbic acid did not undergo any appreciable change either in electrophoretic mobility or in optical properties and enzymatic activity.

3. Discussion

Robinson et al. [6] have shown that treatment with L-ascorbic acid accelerates deamidation of model peptides and of transferrin; it seems therefore reasonable to suppose that deamidation is responsible for the appearance of multiple forms in L-ascorbic-acid-treated α aspartate aminotransferase. The similarity between the electrophoretic, spectral and catalytic properties of the artificially induced subforms and of the subforms occurring in pig heart homogenates [1] suggests that deamidation may also be responsible for the production of the latter.

John and Jones [5] have recently presented evidence, based upon electrophoretic and titrimetric data, supporting this hypothesis.

However no direct chemical data are presently available to prove this point: the small difference in the amide content of the natural α- and γ-subforms reported by Martinez-Carrion et al. [1] cannot be considered significant because of the limited precision of the method. Moreover, according to Banks et al. [3] the differences in the electrophoretic mobility of the naturally occurring multiple forms can also be explained by conformational changes. The importance of conformational factors in causing the lack of activity of the enzyme species characterized by a coenzyme absorption band at 340 nm is demonstrated by the partial activation undergone by this species when submitted to extensive denaturation in 6 M guanidine followed by renaturation [7].

Present evidence could be provisionally interpreted assuming that cytoplasmic aspartate aminotransferase can undergo progressive deamidation and that the enzyme forms which have undergone more extensive deamidation are more susceptible to conformational changes which affect coenzyme binding, and, consequently, activity.

In this respect it is interesting to recall that the controlled deamidation of a protein molecule, modulated by favourable sequences of amino acid residues, has been proposed as a possible timer for development and ageing [8,9].

Concerning the mechanism of induction of multiple forms by L-ascorbic acid, Robinson et al. [6] already suggested that auto-oxidation products of L-ascorbic acid, rather than L-ascorbic acid itself, were responsible for the increased rate of deamidation in the aerobic solutions of transferrin.

The generation of superoxide anions, hydrogen peroxide and hydroxyl radicals during the oxidation of L-ascorbic acid in aerobic solution [10,11], as well as during the course of other oxidative processes [12] has been clearly demonstrated.

A participation of free radicals in the generation of multiple forms seems demonstrated in our case by the slower rate of formation of anodic components observed at low pH, where inactivation of superoxide anions is faster, or in the presence of superoxide dismutase and catalase, which catalyze the inactivation of these radicals.

It is promising, at least as a working hypothesis, to verify the relationship between the L-ascorbic acid-induced production of multiple forms and their natural occurrence.

The possibility of free radicals playing a role in a number of biologically important processes has been suspected since the demonstration [13] that the hydroxyl radical is the chemical species actually responsible for the production of ethylene from methional, a factor which controls the rate of ripening of fruit.

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